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MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF REPRODUCTION OF THE STONE MARTEN, *MARTES FOINA* (MAMMALIA, CARNIVORA), IN THE STEPPE ZONE OF THE SOUTH OF UKRAINE

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Morphological and Physiological Characteristics of Reproduction of the Stone Marten, *Martes foina* (Mammalia, Carnivora), in the Steppe Zone of the South of Ukraine. Steklenev, E. P. — The basic processes of the physiology of reproduction of the stone marten (*Martes foina* Erxleben, 1777) in the steppe zone of south Ukraine were investigated. They were found to occur mainly within the biological cycle of reproduction of this species, distributed in different geographical latitudes. It is characterized by a long period of gestation length (243–263 day) with a long latent period (June–July — end of February) and relatively short (15–16 months) period of actual pregnancy (late February–April). Termination of embryonic development is mainly on the stage of blastula. Implantation of embryos begins in late February, accompanied by processes of gastrulation and the formation of the amniotic membranes. Activation of spermatogenesis in adult males begins in late February and March, the formation of full-mature spermatozoa — in April. Males and females reach reproductive maturity mainly in the age of two years. A slight shift of individual processes of reproduction of the stone marten in the steppe zone of Southern Ukraine to earlier periods, compared with such of the northern latitudes, are due to more favorable climatic conditions of residence.

Key words: Stone marten, south Ukraine, reproduction, gametogenesis, germ, latent period, pregnancy, offspring.

Морфофизиологические особенности размножения каменной куницы, *Martes foina* (Mammalia, Carnivora), в условиях степной зоны юга Украины. Стекленёв Е. П. — Изучены основные процессы физиологии размножения каменной куницы (*Martes foina* Erxleben, 1777) в степных условиях юга Украины. Установлено, что они проявляются в основном в пределах биологического цикла воспроизводства этого вида животных, распространённых в различных географических широтах. Для *M. foina* характерен продолжительный период беременности (243–263 дня) с длительным латентным периодом (июнь — июль — конец февраля) и относительно коротким периодом (1,5–1,6 месяца) собственно беременности. Приостановка развития зародышей происходит в основном на стадии бластулы; их имплантация — в конце февраля, сопровождаясь процессом гастрюляции и образованием околоплодных оболочек. Активизация сперматогенеза у взрослых самцов начинается в конце февраля — марте, образование полноценных спермиев — в апреле. Половой зрелости самцы и самки достигают, в основном, в двухлетнем возрасте. Незначительное смещение отдельных процессов воспроизводства каменной куницы в условиях степной зоны юга Украины на более ранние сроки, по сравнению с такими в северных широтах, обусловлены более благоприятными климатическими условиями их существования.

Ключевые слова: каменная куница, юг Украины, размножение, гаметогенез, зародыши, латентный период, беременность, потомство.

Introduction

Animal reproduction is the point of interest in biological sciences. While researching, much attention is attracted to process of embryonic development, particularly embryonic diapause in passing certain types of mammalian pregnancy. To study this question the representatives of Mustelidae family are the most perspective. In the American mink, for example, the latent period is short and pregnancy lasts about 40 days;

in the forest otter, badger, wolverine, marten, and ermine it is extended and varies within 69–268 days; in the weasel the latent period is absent and a pregnancy lasts approximately 54–56 days (Danilov, Tumanov, 1975). The animals of that family, according to D. V. Ternovsky (1977), are interesting for its mono- and polyestrusity, diversity of sexual cycle, wide fluctuations in periods of active ova- and spermatogenesis. Some of them (weasel, solonhoy), under the certain habitat conditions, are able to breed 2–3 times in one calendar year. The author also noted very early puberty in ermine females; the latter can be paired with an adult male already at the age of 36–65 days, and the next spring bear full offspring. According to P. I. Danylov and I. L. Tumanov (1975), some females of wolverines, badgers and otters do not breed every year, mainly due to food resources.

Some members of this family differ in terms of sexual maturity, mating, bearing offspring and multiple pregnancies. The forest polecat, European and American mink are distinguished by the early timing of puberty; it starts at 11–12 months. The weasel and ermine become sexually mature on the second year of life and mate in early summer. Other members of this family (wolverine, badger, otter, marten forest) reach puberty mainly at the age of three years, although some well-developed females, under favourable climatic conditions, may show sexual activity and mate already at the age of two years (Danilov, Tumanov, 1975; Ternovsky, 1977).

Quite clearly the difference in some Mustelidae species is observed in terms of sexual activity display and rutting period. In species, where pregnancy goes without diapause or short diapause, rut takes place in early spring; in females with prolonged diapause — in early spring or mid summer. Taking into account the fact that the offspring of all members of this family are born underdeveloped, such adaptation allows pregnant females safely survive hard winter and bear offspring in favorable — warm season (Asdell, 1946; Marshall, 1960; Novikov, 1962, 1971; Danilov, Tumanov, 1975; Audy, 1976; Mead, 1989; Sandell, 1990). The starting point of reproductive system activation in most of them is the body response, the hypothalamic-pituitary system in particular, to the change of the length of daylight and the light spectrum. The length of females' gestation and bearing offspring are regulated mainly with duration of latency period, embryo storage until implantation and intensity of its development after implantation. Judging by terms of mating and birth of offspring, the same character of the sexual cycle occurs in the stone marten (*Martes foina* Erxleben, 1777), which area almost coincides with the forest marten, and due to this, perhaps, their genealogical affinity, what suggests the possibility of crossbreeding (Shtrelyi, 1934).

In Ukraine the stone marten is found in mountainous and lowland forests of Transcarpathia to Chernihiv, Kharkiv and Dnipropetrovsk Region, at least — in the steppe zone. It inhabits rocky ravines, mountain slopes covered with bushes and shrubs. It is often found in old parks, uninhabited stone buildings, attics of premises villages and even cities. In the woods this species masters high hollow trees, flooded forests and shelter belts. As repositories it often uses hollow trees, crack rocks, rocky dumps and others.

Material and methods

Considering relatively wide distribution of the stone marten in the steppe zone of Southern Ukraine and, in particular, in the Biosphere Reserve "Askania Nova", we conducted observations on sexual behavior of these animals, while studying the timing of activation of reproductive processes and features of passing gametogenesis, completeness of generative elements, timing of breeding, length of gestation and bearing offsprings. In the study of spermatogenesis in males the weight of the testes and their appendages in different seasons, the consistency of the stroma, the activity of its passage in a specific period, the presence (or absence) of spermatozoa in the caudal parts of the testes appendages, their concentration, activity, morphological and physiological status, etc were taken into account. In the study of females genitalia the indicators of the uterus development, physiological state of the ovaries, in the presence of pregnancy — the number of embryos or foetuses, character of their development and their location in the uterine horns were taken into account. In the study the number of ovarian follicles with the definition of stage of their development, the presence of cyclic corpora lutea or corpora lutea of pregnancy, the number of embryos were counted. More recently potential multiple pregnancy was determined; true multiple pregnancy was determined by the actual number of born offsprings. Age of animals was determined by their common development, the development of dental system, reproductive organs, including copulative organ in males (Ternovsky, 1977; Marshall, 1966). During the development stage and calcification of bakulum (os penis) the terms of reaching their sexual and physiological maturity were determined. Total for research period genitals of 27 individuals, including 15 males and 12 females were studied. These parameters were analyzed in seasonal and age aspects.

Results and discussion

Reproduction of the stone marten in Southern Ukraine, as in other areas of its distribution, has a pronounced seasonal pattern (Danilov, Tumanov, 1978; Audy, 1976). Maxi-

mum performance of sexual activity and fertile pairings takes place in late spring — early summer. During this period, they utter frequent mewling like sounds, searching for breeding partners and mating. This period is characterized by intensive processes of preparing of uterine horns mucous membrane to the adoption of embryos, their nidation and further development.

Gonads of all adults stone martens, studied in this period, were found in the state of activation of hormonal processes that outwardly expressed in the behavior of animals. In the males it appears much earlier (late February — March) and is expressed in activation of spermatogenesis, the intensity of its passing (table 1).

In the saminfarous of testes active division of germ cells begins; they gradually maturing, fill tubules cavity, markedly increasing their diameter. In March spermatogenesis reaches the stage of spermatids, with significant increasing of the testes mass. Maximum size they reach in April, when a large number of normally developed, actively moving sper-

Table 1. Indications of gonad development and passage of spermatogenesis in males of the stone marten

Таблица 1. Показатели развития гонад и прохождения сперматогенеза у самцов каменной куницы

Date of investigation	Age of animals	Weight of the testes (right/left), g		Indicators of spermatogenesis
		total	including appendages	
11.01.99	adult	1.060 (0.490/0.570)	0.236 (0.112/0.124)	Spermatozoa not found neither in appendages of testes, nor in scrape of their stroma
17.01.74	adult	1.00 (0.5/0.5)	—	— « —
19.01.81	adult	0.800 (0.400/0.400)	0.205 (0.096/0.109)	— « —
21.01.70	adult	1.00 (0.500/0.500)	0.300 (0.150/0.150)	— « —
28.01.81	adult	0.800 (0.400/0.400)	—	— « —
29.01.93	adult	0.652 (0.330/0.322)	—	In scrape of testes stroma and their appendages spermatozoa was not detected
15.02.69	adult	1.0 (0.5/0.5)	0.4 (0.2/0.2)	A marked activation of spermatogenesis, the latest achieves the spermatocytes of first order
25.03.98	adult	2,9 (1,500/1,400)	-	Spermatogenesis achieves the stage of spermatids
23.04.69	adult	4.2 (2.1/2.1)	0.7 (0.35/0.35)	In appendages of testes many active spermatozoa
27.06.72	1 year, 2–3 months	0.230 (0.130/0.140)	0.104 (0.056/0.048)	Spermatozoa absent, spermatogenesis reaches the stage of spermatocytes of first order
27.06.78	1 year, 2–3 months	0.297 (0.164/0.133)	0.143 (0.084/0.059)	— « —
4.07.82	1 year, 2–3 months	0.390 (0.180/0.210)	0.155 (0.07/0.085)	— « —
13.11.78	2 years, 4–5 months	0.610 (0.250/0.360)	—	Spermatogenesis in depressive state
7.12.66	adult	1.05 (0.50/0.55)		— « —



Fig. 1. Spermatozoa of male, examined in April. Phase contrast. 280 \times .

Рис. 1. Спермии самца, исследованного в апреле. Фазовый контраст. 280 \times .

matozoa present (fig. 1). On their "juvanility" the presence of the protoplasmatic drop in the cranial part of the flagellum, clipped at the base of the head indicate. As far as spermatozoa maturation the protoplasmatic drop gradually moves to the distal part of the flagellum, and pulls off in physiologically mature spermatozoa. During this period males behave quite actively and ready to mate.

Duration of mating season in the stone marten, judging by the sexual behavior of mature individuals of both sexes and the passage of their gametogenesis, is limited mainly whith June and July. In 3 males, examined in October–December, the presence of sperm in the caudal parts of the appendages and the scraping of testes stroma only remnants of degenerated spermatozoa were found. The latter served as nutritious substrate for anaerobic microorganisms, that penetrated in the seminiferous tubules of the testes from the urinary tract. The lack of hormonal — biologically active substances that inhibit its existence contributed rapid development of microflora. The weight of the males' testes, compared to the summer period, decreased almost four times and averaged 940 ± 170 mg, with a range within 610–1160 mg. That it remained during January–February, with an average weight of 900 ± 100 mg, with a range within the 652–1060 mg. Distinct activation of spermatogenesis begins in February and March; the weight of testes of one male, studied in the second decade of February, was already 1.0 g, the other one — studied in the third decade of March — 2.9 g, with the presence of spermatocytes of second-order and spermatids in saminiferous tubules. In males, examined in the third decade of April, in the caudal parts of the testes appendages, already a large number of normal — actively moving spermatozoa, with a total weight of testes — 4.2 g was recorded.

As a result of studying the morphological and physiological characteristics of the structure of stone martens spermatozoa, compared with other members of the family Mustelidae, it was found that they were distinguished by a great length ($78.6 \pm \pm 0.20$ mm) with peare-like form of heads (Steklenev, 1975, 2005). A relatively large head akrosome cap gives them this form; on the edge it is sickle-shaped, uniformly thick (1.7 ± 0.02 mm) along the length (7.0 ± 0.03 m). A relatively long ($133.3 \pm$

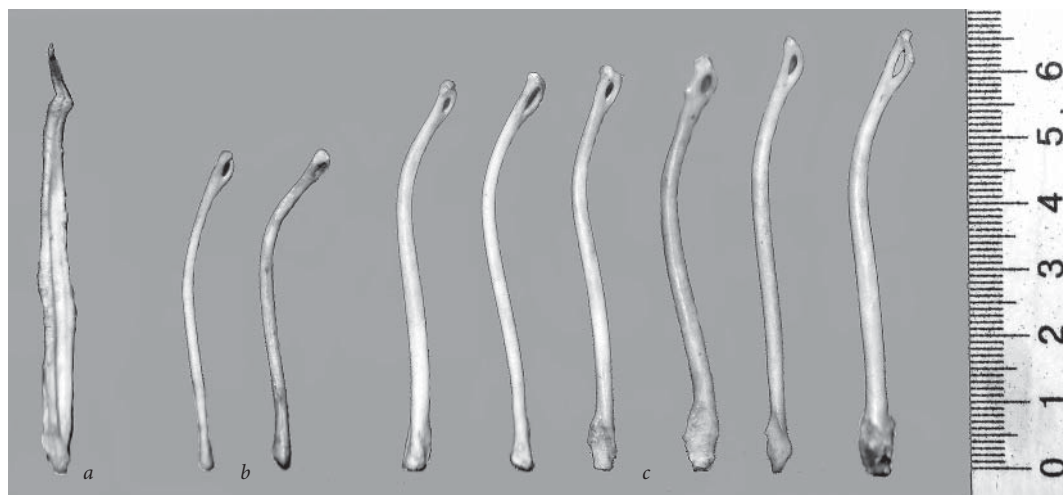


Fig. 2. Baculate rods of copulative organ of males: *a* — in age of one year; *b* — two years; *c* — in adulthood.

Рис. 2. Стержневые косточки копулятивного органа самцов: *a* — в годичном; *b* — в двухгодичном; *c* — во взрослом состоянии.

± 0.09 mm) connective part by its thickness (1.5 ± 0.01 mm) markedly contrasts with subtle and disproportionately long (57.2 ± 0.23 m) main part of the flagellum. Relatively short (2–3 microns) final part of flagellum is unperseptible. Protoplasmatic drop decreases during the ejaculation.

In studies of young male tastes (about 15 months of age) in late June, no spermatozoa were found, but spermatogenesis was already at spermatocyte stage of first order. The weight of testes was 230 mg. Formation of copulative organ bakulum (os penis) was on the stage of cartilage (fig. 2) Spermatogenesis of the testes of two adult males, examined in the first decade of October and in the second decade of December, was clearly in the depression, although in the first case in the appendages the presence of residues degenerated spermatozoa was still noticed. Weight of the testes also markedly decreased and was, respectively, 1.16 and 1.05 g. Such a state of spermatogenesis was seen in the male, examined in the second decade of November at the age of 2 years 4–5 months. The weight of its testicles was only 0.610 g; bakulum was completely calcified, although still small in a size (fig. 2).

The data of our study of sexual activity of stone marten males and intensity of their spermatogenesis were matching those of Yu. S. Lobachov (1973), obtained in a study of males of this species, inhabiting the mountains of South-East Kazakhstan. According to his data, spermatogenesis in males of this population begins in late March, with its gradual acceleration to mid summer, while increasing weight of testes. Maximum size they reach in May and June, the period of increased sexual activity during the mating season. In August the weight of testes starts to reduce markedly, indicating a slower passage of spermatogenesis, and hence the end of rut. Registered by D. V. Ternovsky (1977) cases of rut, and in particular estrous periods in females of the stone marten in Western Siberia lasted from July, 4 to August, 10. According to the V. I. Abelentsev (1973) report, the mating period, of stone martens — “bilodushky”, which was held at the experimental centre “Teremky” (Kyiv Region), also occurred in the third decade of June and lasts until mid of August.

Almost similar figures of reproductive ability was marked in the forest marten (*Martes martes*), disseminated in the same geographical areas as the stone marten. According to P. I. Danylova and I. L. Tumanova (1972), activation of spermatogenesis in adult males of the forest marten also begins in spring (February–March); in the seminiferous tubules of testes of all males examined in this period, they noted the presence of spermatids, in May — already mature spermatozoa. Focusing on these figures, the authors admit that the

Table 2. Condition of genitals of stone marten females

Таблица 2. Состояние гениталий самок каменной куницы

Date of research	Age of animals	Weight of the uterus, g	Length, cm					Weight of ovaries, g		Ovarian condition	
			vagina	cervix	body	horns		right	left	right	left
10.01.57	adult	1.564	5.1	1.5	1.5	4.2	3.3	0.058	0.059	3 corpus luteum of pregnancy, 5 small follicles	2 corpus luteum of pregnancy, 7 small follicles
			pregnant: in the right corner — 3 in the left — 2 blastula								
18.03.68	second year	0.800	3.9	0.4	—	5.0	4.3	0.030	0.030	presence in both ovaries small follicles, normal and under atresia	
26.03.82	adult	62.0 (with fetuses)	pregnant, in each of uterine horns one fetus					0.048	0.036	1 corpus luteum of pregnancy 2 mm in diameter	1 corpus luteum of pregnancy 2 mm in diameter
5.04.05	1 year	1.7	3.8	0.9	—	3.4	3.4	0.025	0.025	availability of prymordial and cavitary follicles	
25.04.69	adult	1.6	4.7	0.8	—	2.8	3.5	0.035	0.040	1 follicle diameter 0.8 mm	1 follicle diameter 1.2 mm, 1–1.0 mm, 3–0.3 mm
18.05.82	1 year	0.8	5.0	1.3	—	3.5	3.5	0.023	0.023	availability of prymordial and cavitary follicles	
4.06.84	2 years	1.1	5.1	1.4	—	5.3	5.5	0.049	0.060	presence of cavitary follicles	
28.06.71	second year	0.84	4.0	—	0.8	2.5	3.2	0.040	0.040	— « —	
10.08.82	adult	1.8	4.9	0.9	0.4	3.2	4.2	0.089	0.091	2 follicles diameter 2–1.5 mm	2 follicles diameter 1–1.5 mm
14.11.78		1.2	3.4	0.4	—	3.0	2.7	0.030	0.028	Presence of follicles and corpora lutea absent	
10.12.67	8 months	—	—	—	—	—	—	0.025	0.025	availability prymordial follicles	
31.12.64	young, 2–3 years	0.840	2.8	0.9	0.3	4.1	4.3	0.040	0.040	1 corpus luteum of pregnancy, 8 small follicles	1 corpus luteum of pregnancy, 7 small follicles
			pregnant, in the right uterine horn 3-blastomer zygote, in the left — blastula 1,230 × 880 mm								

most likely period of runs in the forest marten occurs in the second half of June and the first half of July, although active spermatogenesis lasts until the end of August. The reduction of spermatogenic epithelium was noted in September and December, a period of relative sexual tranquility — in the middle of winter. Males of forest marten, according to the above mentioned authors, reach reproductive maturity at the end of the second — the beginning of the third year of life.

Obvious signs of sexual activity of forest marten females, according to P. I. Danylov, I. L. Tumanov (1976), and I. D. Stroková (1947), also occur in summer; the best time of the runs — the second half of June and July. Duration of pregnancy in fertilized females (including diapause) similar to that of stone marten (Manteufel, 1947), although to a large extent is determined by the geographical area of residence. According to Sokolov (1963),

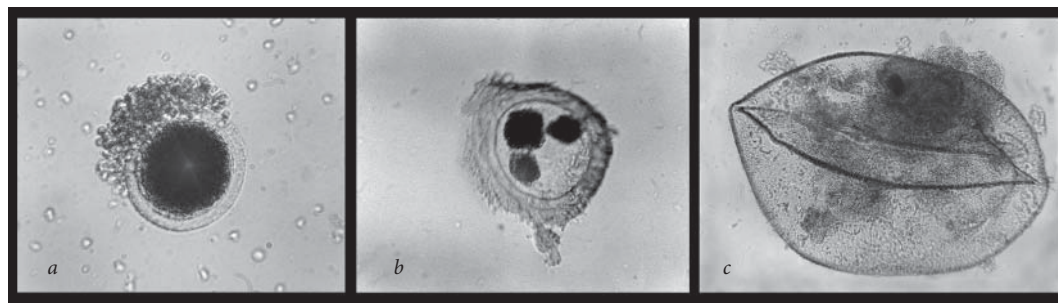


Fig. 3. Ovum (a) and germ of female studied during diapause of pregnancy: b — 3-blastomeres zygote; c — blastula.

Рис. 3. Яйцеклетка (a) и зародыши самки, исследованной в период диапаузы беременности: b — 3-бластомерная зигота; c — бластула.

generalized indicator of female's pregnancy of stone marten ranges within 236–274 days. Considering its relative affinity to the forest marten (Shtreyli, 1934), it can be stated, that some of its processes are in the same sequence, applied to puberty, manifestation of sexual activity, timing of mating, fertilization and duration of pregnancy. Data of our research of detailed study of their genitalia conducted within 5 years indicate this (table 2). Of 12 examined females — 3 were pregnant, other females were unfertilized or at the stage of puberty. Reproductive organs of a young (one year) female were in juvenile condition and weighed only 0.7–0.8 g, including ovaries — 0.024 ± 0.004 g; only primordial and cavitory follicles 250–500 microns in diameter, which gradually degenerated in the early stages of development, were recorded. In the two years old females weight of genitals slightly increased and ranged 0.84–1.1 g, ovary weight — 0.041 ± 0.006 g; small follicles with a diameter of 1.5 mm and a separate atretic body were recorded. In the ovaries of one adult female, studied on the first decade of August, 2–3 follicles 1–1.5 mm in diameter were recorded in each ovary; their weight was 180 mg. The weight of the uterus was 1.8 g, the length of right corner — 3.2 cm; left — 4.2 cm. Judging the state of the genitals, it might give birth this year.

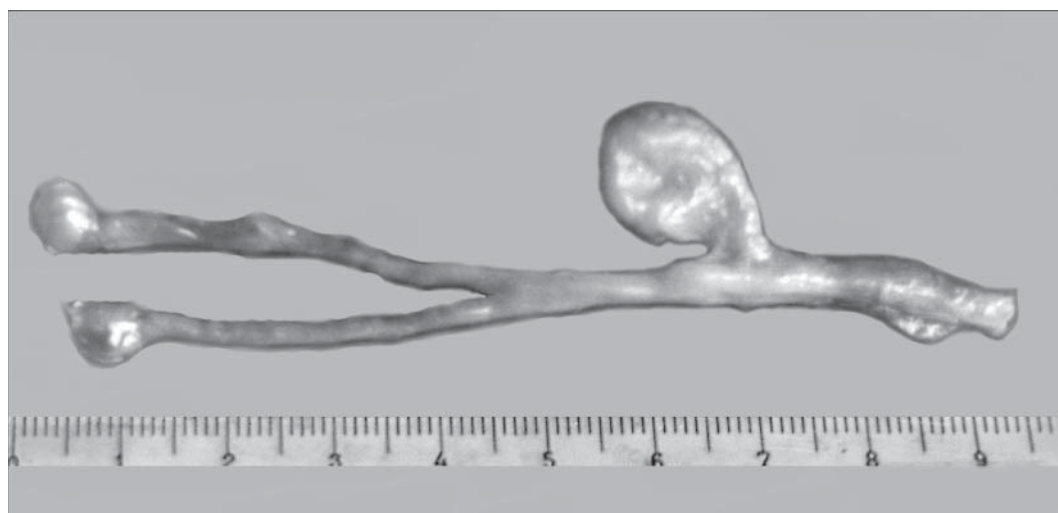


Fig. 4. The uterus of pregnant female, studied 31 December; in the right horn the highlighted location of blastula.

Рис. 4. Матка беременной самки, исследованной 31 декабря; в правом роге выделяется место размещения бластулы.

From December–January the uterus in fertilized females was markedly increased, indicating their training to availability for embryos implantation. In female, studied on December, 31 uterine weight was already 840 mg; the length of the right horn — 4.4 cm; left — 4.6 cm. In the cavity of right horn the presence of one 3-blastomer zygote with diameter of 188.15 μ m, in 1.29 times more than mature ovum ($145.8 \pm 3.1 \mu$ m) was recorded; the diameter of individual blastomeres fluctuated between 50–72 microns with normal yolk granularity, as in mature ovum (fig. 3). In the left horn oval blastula, with length 1230 μ m and width — 880 μ m, with obvious signs of normal development was recorded. The outer wall of the uterine horn in place of blastula nidation was thickened and had a dark brown color (fig. 4). In both ovaries one yellow body (corpus luteum) of pregnancy with a diameter of 2–3 mm and 7–8 follicles diameter 1.5–2 mm was seen; the weight of each ovary was 40 mg. A big difference in the development of two embryos of that female seemed uncommon. We can assume, that marked underdevelopment of 3 — blastomer zygote, found in the right uterine horn, was due to delayed fertilization, which was released probably in postestrual period, possibly as a result of forcible copulation, and stopped its development in the early stages of division, simultaneously with the termination of blastula, that was in the second uterine horn. Maybe, it was early degeneration, although, judging external morpho-physiological characteristics, it developed normally (fig. 3). Characteristic that in this case the dark spot on the outer wall of the uterus horn, — the place of blastula, — meanwhile, was not recorded. Judging the development of the genitalia and the number of embryos, female was pregnant for the first time after puberty.

The data of another adult female investigation obtained at the end of the first decade of January (10 days later) indicate the same state of development of embryos of the stone marten in this period. In the right horn of her uterus the presence of three, in the left — two embryos, which also were in the stage of blastula with obvious signs of normal development were recorded. In her ovaries there was the same number of corpora lutea of pregnancy: in the right — 3, in the left — 2, and, accordingly, — 5 and 7 follicles with a diameter of 1.5 mm. The weight of uterus with embryos was 1,564 mg what is almost twice the previous female (840 mg); the length of blastulas averaged 1,210 μ m, width — 685 microns. In the cavity of all the blastulas the embrioblasts were already seen. Judging the morphological features of separate blastulas, they developed synchronously, evenly distributed along the length of uterine horns. The places of embryos from the outside wall of the uterine horns were also distinguished by brown tint and slight thickening of tissue. It should be mentioned that all embryos of both females were in close organic contact with the parent body — uterine wall, to which they were “tied” with bunch of tissue fibers, by which received nutrients to sustain their viability.

Indicators of research in female genital on the third decade of March can tell about the nature of development of stone marten embryos in Southern Ukraine. Weight of uterus with foetuses and embryonic fluid equals 62 g. In the cavity of uterine horns was marked presence of two foetuses: in the right — male, length 8.4 cm and weight 19.6 g; in the left — female, length of 8.0 cm and weight 15.5 g. Weight of ovarian equal, right — 48 mg, left — 36 mg. Each of them is marked by one of active yellow body of pregnancy, 2 mm in diameter and 1–2 follicles with a diameter of 1.5 mm.. The surface of the foetuses body was pinkish and had short hairs on the back and sides of the torso. Judging these figures, the foetuses developed normally and were already at a late stage of embryogenesis. Considering the actual duration of “proper” pregnancy of this species, that is equal to one and a half of month (Danilov, Tumanov, 1976), it may be asserted that the implantation of embryos of females occurred sometime in the third decade of February. To that period the endometrium and, uterine glands were preparing to their perception. It can be asserted also, that, the given parameters of embryos, the birth of this female should take place on the first week of April. Apparently, according to our

observations and reports of hunters, deliveries in females of stone marten in Southern Ukraine are mainly in that period.

Conclusions

Based on the analysis of the main indicators of the physiology of reproduction of the stone marten in Southern Ukraine steppe conditions, we can conclude that they occur mainly within the biological cycle of reproduction of this species in different climatic latitudes. It is characterized by a long "common" duration of pregnancy, (about 243–263 days) and a relatively short period of "actual" pregnancy (about 1.6–1.8 months), with mating in June–July and the bearing offsprings in April–May next year. Termination of embryonic development is under blastula. Implantation of embryos occurs in late February, accompanied by the beginning of gastrulation and the formation of fetal membranes. Apparent activation of spermatogenesis in adult males begins in late February–March, the formation of full-fledged sperm — in April, indicating their readiness to mate. Reproductive maturity of males and females reaches mainly in the age of two years. The slight shift of separate reproduction processes of the stone marten in the steppe zone of Southern Ukraine to earlier periods, compared with the northern latitudes, is due, perhaps, to their more favourable climatic living conditions.

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